

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of)
Pierre DRUILHE) Group Art Unit: 1645
Serial No.:10/774,602) Examiner: N.M. Minnifield
Filed: February 10, 2004)
For: PLASMODIUM FALCIPARUM ANTIGENS INDUCING PROTECTIVE
ANTIBODIES

1.132 DECLARATION

Hon. Commissioner of Patents
P.O. Box 1450
Alexandria, Virginia
22313-1450

*Conradized
9/21/05*
I, Pierre Druilhe do hereby declare the following:

[1] I am currently the director of the Biomedical Parasitology Unit at Institut Pasteur in Paris, France and have been the director of this department for many years. I have published 260 articles and have 10 U.S. Patents issued in my name. I am one of the inventors of the above-captioned U.S. patent application. Enclosed please find attached a copy of my *Curriculum Vitae*.

[2] I have read the last U.S. Official Action dated March 25, 2005, for the above-identified patent application. It is my understanding that the Examiner deems that the claims directed to vaccines cannot be produced by a scientist using the disclosure of the above-captioned specification since the specification does not teach a scientist how to obtain a malaria vaccine. It appears that the Examiner's reasoning in

maintaining this rejection is that she deems that none of the claimed peptides either alone or in combination will protect against malaria. I respectfully disagree with the Examiner for the following reasons.

[3] MSP-3 is a polymorphic protein that binds to the merozoite surface and traffics to the parasitophorous vacuole. It has a molecular weight of 48 KD and is recognized by cytophilic antibodies IgG1 and IgG3 in protected subjects and non-cytophilic antibodies in unprotected subjects. This protein is further capable of inducing antibodies which cooperate with monocytes in the antibody-dependent cellular inhibition (ADCI) reaction. Within this protein, certain conformational epitopes are recognized by T lymphocytes. The present specification provides long synthetic peptides which have particular conformational epitopes recognized by T lymphocytes and B epitopes such as MSP-3a, MSP-3b, MSP-3c and MSP-3d. Furthermore, IFN- γ secretion in response to the LSP peptide was extremely high in the range of 10,000 to 50,000 International Units and remained high over the immunization process.

[4] Besides the overview presented above in paragraph (3), the present specification describes the isolation of the clone DG210. The characterization of the protein synthesized by this clone such as inducing an ADCI reaction *in vivo*, the study of lymphoproliferative responses, the sequencing and characterization of the genome clone, and the isotopic characteristics ; i.e., the protein is recognized preferentially by IgG1 and IgG3 in the blood of protected subjects is also described in the present invention.

[5] The study of the polymorphism of the gene and epitopes defined by the clone DG210 was also undertaken using two cultures of strains of African *P.falciprum*, of 4 Thai isolates and 29 African isolates. Screening by Western blot with 6 Thai or African isolates and indirect immunofluorescence of 10 isolates from the Congo confirmed that the epitopes are representative of non-polymorphic conserved regions. The results of these studies were also described in the specification.

[6] A phase I clinical study using a long synthetic peptide derived from MSP3 to evaluate the safety and tolerance of the vaccine, as well as the immunogenicity was disclosed in the specification. In this regard, 36 Swiss volunteers were administered

the synthetic peptide from MSP-3 with either Alum or Montanide® as an adjuvant. The long Synthetic Peptide formulation of MSP-3 was found to be safe and immunogenic. The antibodies that were induced were IgG1 and IgG3, which subclasses of antibodies bind to the Fc gamma receptors on monocytes, thus indicating the monocyte-dependent, antibody-mediated effect.

[7] Additional studies set forth in the specification show natural passive transfer of antibodies from mother to newborns, of IgG3 antibodies and that IgG anti MSP-3b antibodies strongly differentiated 2 groups. Studies were also undertaken in cerebral malaria patients. It should be recalled that cerebral malaria involves the clinical manifestations of *Plasmodium falciparum* malaria that induces mental status and coma. It is a disease of the brain that is accompanied by fever and has a 25% to 50% mortality rate. Ring-like lesions in the brain are major characteristics of this etiology.

[8] The results of a study in Niakhar with 4,200 children, which were sampled during the non-transmission season and followed during the transmission season was also described in the specification. 51 children acquired cerebral malaria, 9 of which died despite treatment. The 9 children that died had significantly lower titers of anti-MSP-3 antibodies compared with the 42 cerebral malaria children who survived and as further compared with 100 acute uncomplicated malaria cases. A similar study was conducted on patients in a hospital of Dakar except serum was taken upon admission. A significant difference was found in IgG3 antimalarial antibodies between survivors and individuals who passed away; i.e., lower titers of anti MSP-3 antibodies were found in those patients who passed away.

[9] Finally the specification discloses a SCID mouse model infected with *P. falciparum* and shows passive transfer in SCID mice.

[10] Besides, the disclosure in the specification additional studies were undertaken since the patent application was filed. The association of MSP-3 antibodies with protection against malaria was demonstrated in Dielmo with over 2 years of follow-up. In the Dielmo studies over 220 individuals of all ages were followed, as well as 62 pregnancies. Daily medical exams were given and sequential sera were taken (at least 88,000 sera samples) preceding a given malaria attack. It was also

demonstrated in Burma in an hyperendemic area and by immunization and challenge of Aotus monkeys.

[11] In fact, additional Western blots, and SGI% of the volunteers which were previously injected with 3 doses of MSP-3 long synthetic peptides in comparison with P1AG were taken after 1 year. Further measurements were made greater than 1 year after immunization of the MSP-3 LSP's of the present invention. The results of the firsts study are indicated in attached Annex I. As can be seen from these results and our further results of greater than one year, the immune response obtained after immunization was long-lasting. Furthermore, biological assays demonstrated that the induced antibodies can mediate protection against *P. falciparum*.

[12] Further results were undertaken in a volunteer that was Western blot positive and administered the MSP-3 LSP's of the present invention. As can be seen in enclosed Annex II, after 12 months post immunization, the parasitemia dropped to 0.01 % on day 30.

[13] Thus, in view of paragraphs [2] to [12] above, the conclusions that can be made are the following:

- (a) that MSP3-LSP's set forth in the present invention provide a malaria vaccine that is safe, well tolerated when adjuvanted with alum or Montanide® and are able to elicit antibodies in humans that are able to kill *P. falciparum*;
- (b) even low doses of MSP3 LSP's injected with simple adjuvants induce long-lasting antibodies of the cytophilic classes;
- (c) the MSP3 LSP's of the present invention are directed to fully conserved epitopes and have a strong biological effect against *P. falciparum*; and
- (d) the antibodies elicited in volunteers, after injection of the MSP-3 long synthetic proteins of the present invention, were able to inhibit parasite growth both *in vivo* and *in vitro* via the antibody-dependent monocyte-mediated mechanism.

[14] It appears that the Examiner has taken a strict interpretation of the word "vaccine" since at page 7 of the Official Action, the Examiner states that "none of these are clearly indicative that the claimed peptides either alone or in combination will protect against malaria. An immune response is not protection against malaria."

However, it is well known by scientists in this field that the goal of erythrocytic malaria vaccines is either to reduce the parasite load by preventing invasion of red cells or to prevent parasite replication and/or growth after invasion of the parasite. In this regard the present invention provides a mechanism to block the *P. falciparum* parasite as demonstrated in the specification. Thus the result of the ADCI experiments, which is a mechanism mediated by soluble components released by monocytes that block the division of intraerythrocytic parasites, is a clear indication of parasite killing. Indeed, ADCI can provide a means of generating cross-strain protection, since macrophages activated by antibodies to one variant can kill parasites in red blood cells of other strains and variants of *P. falciparum*. Moreover ADCI can continue after merozoite invasion and entry into the red blood cells.

[15] In view of paragraphs [1] to [14], it can only be concluded that a scientist in this field can gather from the contents of the specification that malaria vaccines can be produced by a scientist using the disclosure of the above-captioned specification

I also declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

29-05-2005

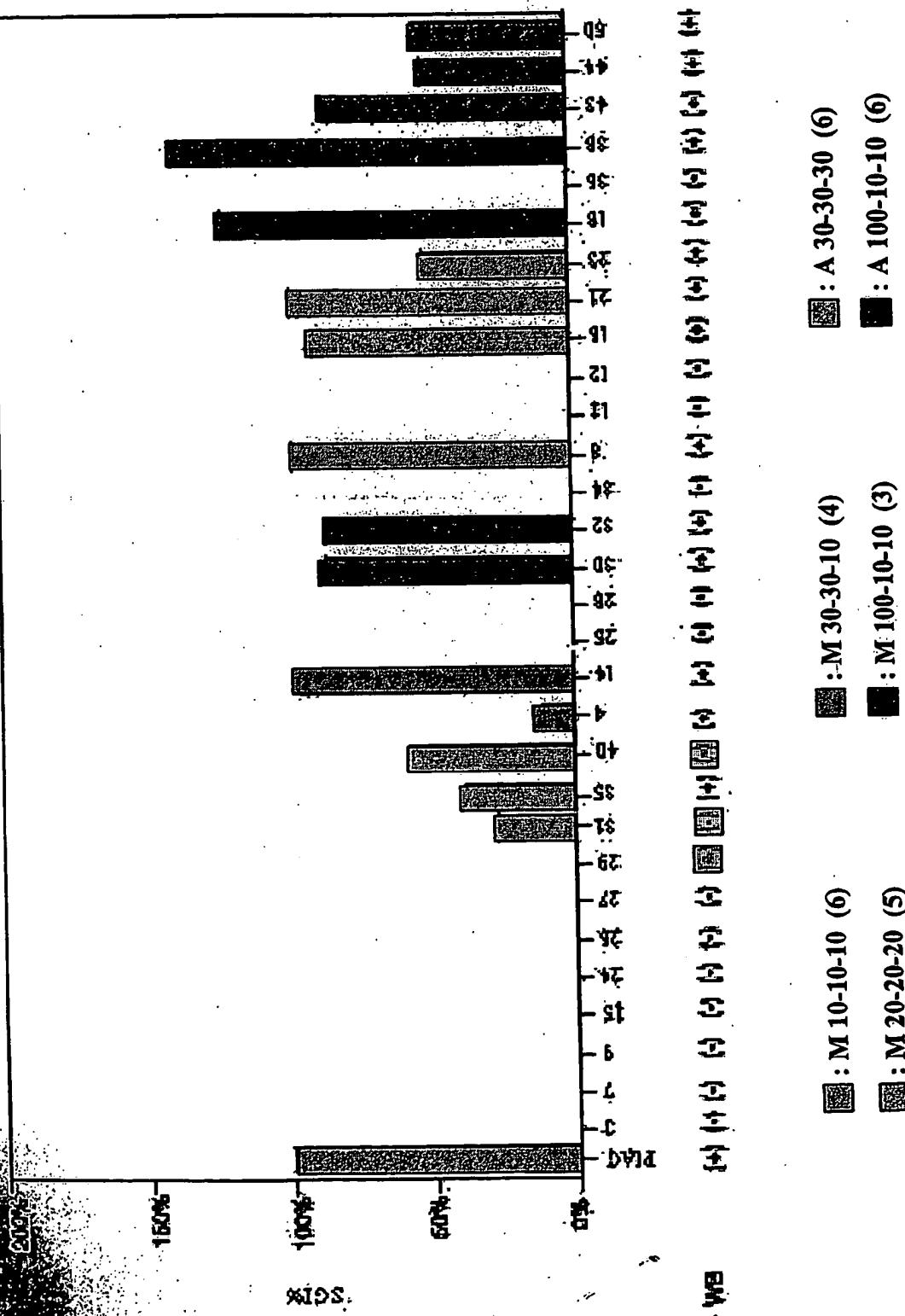
Date



Pierre DRUILHE

Annex I

Western blot result and SGI% of the volunteers one year after 3 doses of MSP3 Long Synthetic Peptide immunization in comparison with PLAG.



Volunteer 18, 12 months post-immunisation, WB+

